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COUMARINS FROM *CLAUSENA INDICA**

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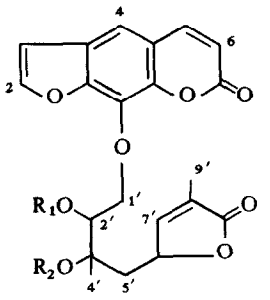
Key word index—*Clausena indica*; Rutaceae; suberosin; pubesinol; pubesinol acetonide; xanthotoxol; 8-hydroxy-5-methoxypsoralen; byakangelicin; indicolactonediol.

The roots of *Clausena indica* Oliv. (Rutaceae) have been studied extensively in the past for furanocoumarins, carbazoles and sesquiterpenes by Joshi and coworkers [1–5]. The reported cardiovascular activity in the crude extracts of this plant in our screening programme prompted us to undertake a reexamination of its chemical constituents [6].

The hexane and EtOAc soluble fractions of the EtOH extract of the aerial portions of the plant after column chromatography over Si gel furnished suberosin, pubesinol, pubesinol acetonide, xanthotoxol, 8-hydroxy-5-methoxypsoralen, byakangelicin and a new C_{10} ether of xanthotoxol named indicolactonediol. The present communication describes essential data leading to the assignment of structure (**1**) to this ether.

Indicolactonediol, mp 116–117° analysed for $C_{21}H_{20}O_8$(M^+, 400); $(\alpha)_D^{25°}$ + 13.3° (*c*, 2.32, MeOH). The UV spectrum λ_{max}^{MeOH} 223, 244, 250, 263 and 303 nm (log ε 4.93, 4.85, 4.87, 4.70 and 4.60 respectively) remained unchanged on addition of alkali and was characteristic of linear furanocoumarin. The IR bands at 3400, 1745 and 1715 cm^{-1} indicated the presence of (i) an OH (ii) an α, β-unsaturated-γ-lactone and (iii) an α, β-unsaturated δ-lactone group in (**1**). Indicolactonediol when treated with C_5H_5N/Ac_2O at room temperature gave a monoacetate (**2**) (M^+, 442), in which one methine proton which appeared originally at τ 5.92 moved to τ 4.75 indicating the secondary nature of the OH group. The IR of (**2**) still showed the presence of an OH group indicating that it was tertiary in nature. This was confirmed by the formation of a diacetate (**3**) (M^+, 484). The vicinal nature of the OH groups was proved by the formation of an acetonide derivative (**4**) (M^+, 440). Indicolactonediol on treatment with 11 M HCl under reflux conditions afforded xanthotoxol indicating that (**1**) is a C_{10} ether of xanthotoxol. The MS of the compound further indicated that the two OH groups, and the α, β-unsaturated-γ-lactone moiety were parts of this C_{10} side chain.

The 100 MHz PMR spectrum of indicolactonediol



(**1**) $R_1 = R_2 = H$
(**2**) R_1 = Ac, R_2 = H
(**3**) $R_1 = R_2$ = Ac
(**4**) R_1 and $R_2 = CMe_2$

integrated for 20 protons. The protons at C-2 and C-3 in the xanthotoxol nucleus appeared as a pair of doublets at τ 2.29 and 3.17 (J = 2.2 Hz). Another characteristic pair of doublets appeared at τ 2.24 and 3.63 (J = 10.12 Hz) and was assigned to the C-5 and C-6 protons of the coumarin nucleus. As expected the C-4 proton was observed as a singlet at τ 2.6. A pair of double doublets of two protons which appeared at τ 5.49 (J = 6.6 and 10.25 Hz) and 5.24 (J = 4.64 and 10.25 Hz) was assigned to the OCH_2 group. The 2′ methine proton appeared as a double doublet at τ 5.92 (J = 6.6 and 4.64 Hz) and the 5′ methylene group was observed as a multiplet at τ 7.88. A multiplet centred at τ 4.73 was assigned to the 6′ proton of a 5 membered lactone. The triplet at τ 8.08 (J = 1.7 Hz) for 3 protons could be assigned to the only remaining Me function at the α-position of the α, β-unsaturated-γ-lactone group, the multiplicity being explained by the assumption of a long range coupling (homoallylic) with the 6′ proton. The corresponding β-proton appeared at τ 2.81 as a multiplet. A 3 proton singlet at τ 8.58 was assigned to a Me on a tertiary OH group. Two exchangeable protons for OH groups were observed at τ 6.67 and 6.88.

In the INDOR experiment (90 MHz; Perkin-Elmer R-32 instrument) when the monitoring field was set at τ 8.08, a sharp singlet appeared at τ 2.75 (H-7′) together with a broad peak at τ 4.73 (H-6′) establishing the relationship of H-9′ with H-6′ and H-7′; conversely setting the monitoring field at τ 2.78 caused a broad signal to appear at τ 4.73 (H-6′) and another signal at τ 8.08 due to H-9′. The interaction among H-6′, H-7′ and H-9′ was further substantiated by a decoupling experiment. Irradiation at τ 8.08 caused the multiplet at τ 2.81 to change to a doublet accompanied by a simultaneous change in shape of the multiplet at τ 4.73. Likewise, the irradiation at τ 2.81 resulted in the collapse of the triplet at τ 8.08 to a doublet followed by a change in shape and multiplicity at τ 4.73.

The relationship between H-5′ and H-6′ was also proved by a spin decoupling experiment. Irradiation at τ 4.73 (H-6′) caused the triplets at τ 8.08 to collapse to a doublet and at the same time a change in the shape and multiplicity of the methylene at τ 7.88 and H-7′ at τ 2.81 were also observed. The relationship between H-2′ methine and H-1′ methylene which constituted a 12 line spectrum of an ABX system was also confirmed by irradiating at τ 5.92 (H-2′) when the double doublet at τ 5.24 (H-1′) collapsed to a doublet. The effect on another double doublet at τ 5.49 (H-1′) could not be recorded.

The MS data given in the experimental section and discussed schematically in Fig. 1 for the fragmentation pattern of indicolactonediol were consistent with the proposed structure. These data together with those obtained from PMR and IR suggested structure (**1**) for indicolactonediol.

* CDRI Communication No. 2335.

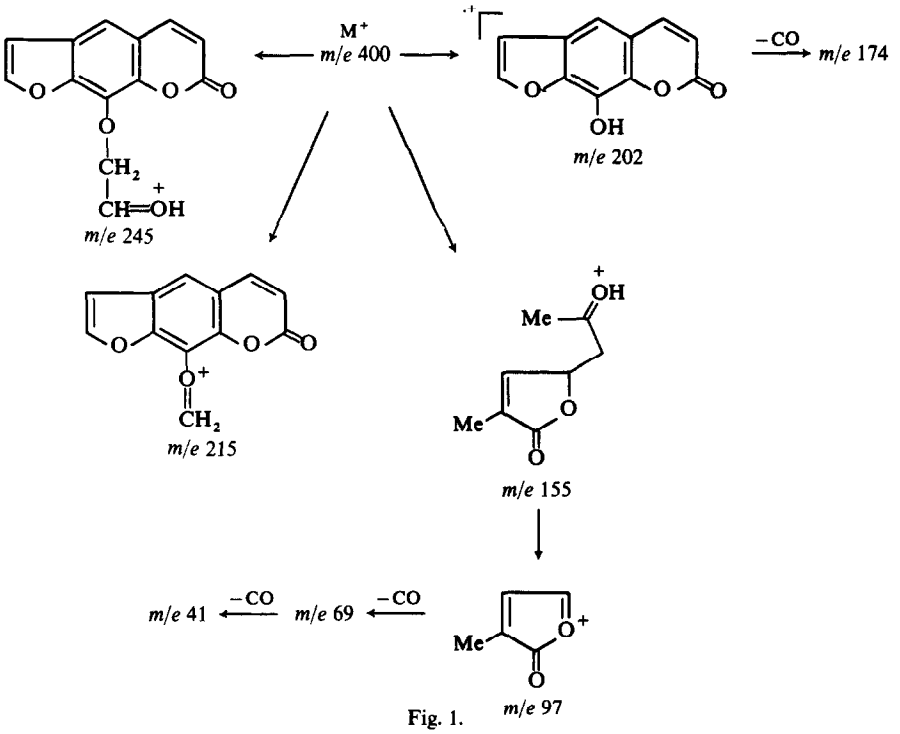


Fig. 1.

EXPERIMENTAL

The ground and dried aerial parts of *C. indica* (1.75 kg) were extracted with 95% EtOH (10 l.). The EtOH extract was concentrated *in vacuo* and macerated with *n*-hexane (3 × 300 ml) followed by EtOAc (4 × 300 ml). The combined *n*-hexane extract was washed with H_2O, dried and the solvent removed. The residue (6 g) was chromatographed on a column of Si gel (200 g) to afford suberosin (210 mg) on elution with *n*-hexane–C_6H_6. Similarly the EtOAc residue (17.4 g) after column chromatography on Si gel (700 g) afforded pubesinol acetonide (150 mg), xanthotoxol (180 mg), 8-hydroxy-5-methoxypsoralen (100 mg), pubesinol (400 mg), byakangelicin (20 mg) and indicolactonediol (300 mg) with a mixture of C_6H_6–EtOAc as eluant.

Indicolactonediol (**1**). mp 116–117° $(\alpha)_D^{25°}$ +13.3° (*c*, 2.32, MeOH), MS: *m/e* 400 (12%), 245 (6), 232 (6), 215 (4), 203 (18), 202 (100), 174 (14), 155 (15), 97 (7.5), 95 (8), 78 (75), 77 (18), 69 (7), 59 (11), 57 (10), 52 (27), 43 (25) and 41 (23).

Indicolactonediol monoacetate (**2**). A soln of (**1** (30 mg) in C_5H_5N (1 ml) and Ac_2O (2 ml) was allowed to stand 18 hr at room temp to give (**2**) , (25 mg); PMR ($CDCl_3$) τ 8.55 (*s*, 3H, Me, H-4'), 8.1 (*t*, 3H, Me, H-9'), 7.95 (*db*, *J* = 6.5 Hz, 2H, H-5'), 7.85 (*s*, 3H, OAc), 7.34 (*bs*, 1H, exchangeable with D_2O), 5.44 (*dd*, *J* = 6 and 11 Hz, 1H, H-1'), 5.09 (*dd*, *J* = 4 and 11 Hz, 1H, H-1'), 4.75 (*m*, 2H, H-2' and H-6'), 3.75 (*d*, *J* = 10 Hz, 1H, H-6), 3.29 (*d*, *J* = 2 Hz, 1H, H-3), 2.78 (*m*, 1H, H-7'), 2.72 (*s*, 1H, H-4), 2.39 (*d*, *J* = 2 Hz, H-2) and 2.32 (*d*, *J* = 10 Hz, 1H, H-5); MS: *m/e* 442 (16%), 282 (2.5), 241(34), 203(17), 202(100), 181(15), 174(25), 145(34), 121(76.5), 97(29) and 69(51).

Indicolactonediol diacetate (**3**). A mixture of (**1**) (20 mg) in C_5H_5N (1 ml) and Ac_2O (2 ml) was heated at 100° for 4 hr to furnish (**3**) (20 mg); PMR ($CDCl_3$): τ 8.23 (*s*, 3H, Me, H-4'), 8.1 (t, 3H, Me, H-9'), 7.95 (s, 3H, OAc), 7.85 (*s*, 3H, OAc), 7.59 (*bd*, *J* = 6.5 Hz, 2H, H-5'), 5.49 (*dd*, *J* = 6 and 11 Hz, 1H, H-1'), 5.1 (*dd*, *J* = 4 and 11 Hz, 1H, H-1'), 4.78 (*m*, 1H, H-6'), 4.29 (*m*, 1H, H-2'), 3.79 (*d*, *J* = 10 Hz, 1H, H-6), 3.2 (*d*, *J* = 2 Hz, 1H, H-3), 2.8 (*m*, 1H, H-7'), 2.76 (*s*, 1H, H-4), 2.37 (*d*, *J* = 2 Hz, 1H, H-2) and 2.3 (*d*, *J* = 10 Hz, 1H, H-5); MS: *m/e* 484 (10%), 283 (60), 241 (12), 223 (24), 202 (50), 199 (10), 181 (24), 167 (49), 163 (20), 155 (30), 149 (100), 141 (71), 113 (45), 111 (33) and 97 (57).

Indicolactonediol acetonide (**4**). A suspension of (**1**) (5 mg), Me_2CO (5 ml) and anhydrous $CuSO_4$ (10 mg) was stirred at room temp. for 18 hr to afford (**4**) (5 mg), mp 110°: MS: *m/e* 440 (16%), 425 (11), 383 (6), 329 (4.5), 299 (10), 269 (11), 245 (12), 239 (17), 203 (31), 202 (100), 201 (14), 189 (14), 181 (9), 174 (10), 163 (10), 149 (15), 131 (13), 111 (11) and 97 (18).

Cleavage of indicolactonediol with HCl. A suspension of (**1**) (50 mg) in 11 M HCl (15 ml) was refluxed for 1 hr, cooled, diluted with H_2O (20 ml) and extracted with Et_2O (5 × 20 ml). The Et_2O layer was washed with H_2O, dried (Na_2SO_4) and the solvent removed to afford the crude product (18 mg) which was purified by PLC on Si gel to yield xanthotoxol (8 mg), mp 248–251°.

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